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Publication date:
2019

Document Version
Peer reviewed version

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Citation (APA):
Hasanzadeh, A., Junicke, H., & Gernaey, K. V. (2019). *Electrochemical biosensors for in-situ monitoring of stress responses in large bioreactors*. Abstract from 5th International Conference Implementation of Microreactor Technology In Biotechnology, Cavtat, Croatia.

Electrochemical biosensors for in-situ monitoring of stress responses in large bioreactors

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Keywords: On-line monitoring, Fermentation, Electrochemical sensors, Glucose biosensors, Freely floating sensor

Monitoring of fermentation processes is considered complex due to the intricate nature of biological systems and their interaction with the surrounding physical and chemical environment. In a bioprocess, metabolite concentrations vary with the different fermentation phases. Non-ideal mixing conditions further contribute to the formation of local concentration gradients. These conditions render process monitoring a challenging task, specifically in large bioreactors.

The efficiency of bioprocesses critically depends on the precise control of cultivation parameters. Controlling parameters like dissolved oxygen, pH, pressure, and temperature is often insufficient to reduce the variability in the process. In fact, factors like nutrient concentrations, growth balance, and intracellular metabolic products are all relevant for understanding the process state. Therefore, the sensing techniques employed need to be rapid to facilitate this deeper understanding of the process. Rapid quantification is also required for improved process control. For example, online monitoring of key primary and secondary metabolites enables fast decision-making through applying dynamic feeding strategies that are tailored to the process conditions.[1] Besides the quick response, electrochemical sensors offer further advantages over conventional methods, such as high specificity and simplicity of design. Combining online process monitoring with automation, it is possible to initiate process corrections with feedback control.[2] However, with offline analysis, these corrections can be implemented only retrospectively. Online monitoring is also key to observing early warning signals based on process deviations, which can be corrected by appropriate control strategies. In fact, with these insights, it is possible to achieve higher productivities, e.g. by monitoring glucose and glutamine levels and by maintaining the balance of these carbon and nitrogen sources, such that animal cell cultures can be run at optimal conditions.[3]

Results obtained in our lab demonstrate that the determination of glucose levels is an important prerequisite for fermentation process control, and that electrochemical biosensors are the easiest and cheapest way of monitoring.[4] In this work, we present the data obtained by continuous online monitoring of glucose concentrations in lab-scale yeast fermentations. These measurements were performed by an integrated amperometric glucose biosensor. The reliability of such sensors was initially confirmed in synthetic samples. Figure 1(a) compares the sensor calibration curve obtained with pure glucose solutions (0 - 0.54 g/L multiplied by 50 in order to be in the range of glucose concentration in real fermentation samples) to a two-point calibration obtained in yeast peptone dextrose (YPD) medium spiked with 20 g/L of glucose. Sensor performance remained stable even with the complex fermentation medium. The sensor was then challenged in a real fermentation process. Figure 1(b) shows results of the on-line monitoring of glucose concentrations during *S. cerevisiae* growth for 5 h. The sensor tracked glucose levels with high accuracy and time resolution. A slow decrease in substrate concentration was observed during the first 3 h of growth, followed by a fast decline in the last 1.5 h. Due to the low detection limit of the sensor, a very accurate determination was possible even close to glucose depletion.

Obtained results set the frame for an innovative measuring concept which be presented by the authors. In a traditional bioreactor set-up, data about each measured variable is collected by a single sensor that is mounted in a fixed position close to the reactor wall. In the novel concept, miniaturized biosensors are mounted on free-floating sensor particles (developed by the SME FreeSense ApS). Data are transmitted by wireless technology as the sensor particle surfaces the fermentation broth. As the authors wish to illustrate (Figure 2), this emerging technology offers great potential for accurate on-line monitoring of fermentation processes. An outlook is given on how novel electrochemical biosensors will contribute to monitoring stress responses in large scale bioreactors.

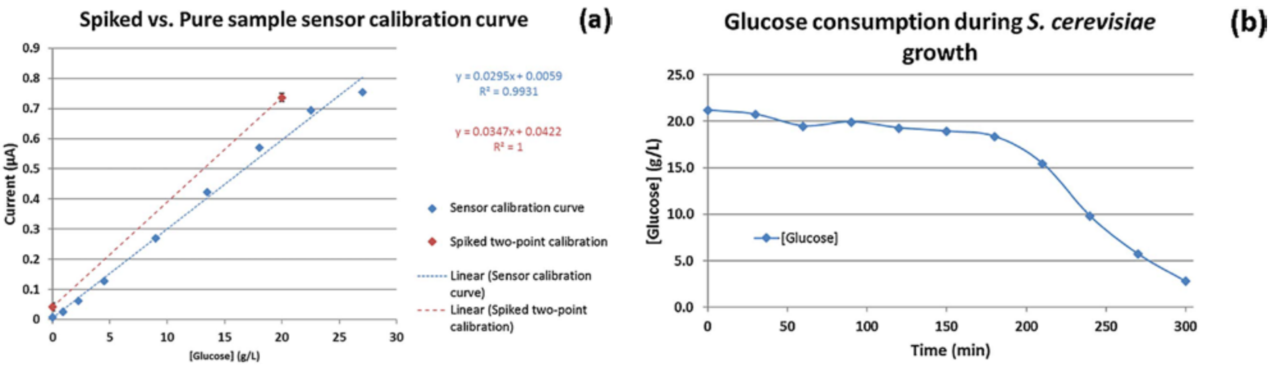

Figure 1. Graphite sensor performance with fermentation samples: (a) pure sample calibration curve (dotted line) vs. glucose spiked YPD media two-point calibration (dashed line) and (b) on-line quantification of glucose consumption by *S. cerevisiae* in a micro-bioreactor determined by a glucose biosensor (N = 3). Adopted from Fernandez et al.[4]

Figure 2. Freely floating sensor particle (courtesy of FreeSense ApS)

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